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Award Number: DAMD17-01-1-0152

TITLE: Lymphatic Regeneration Within Porous VEGF-C Hydrogels for Secondary Lymphedema

PRINCIPAL INVESTIGATOR: Mauricio A. Contreras, M.D.

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center
Boston, MA 02215

REPORT DATE: January 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-01-2006	Final	1 Jul 2005 – 31 Dec 2005

4. TITLE AND SUBTITLE
Lymphatic Regeneration Within Porous VEGF-C Hydrogels for Secondary Lymphedema

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-01-1-0152

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Mauricio A. Contreras, M.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Beth Israel Deaconess Medical Center
Boston, MA 02215

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT
Introduction: Lymphedema is an abnormal swelling, in which lymph production exceeds drainage capabilities. This occurs as a result of lymphatic vesdestruction during the removal of lymph nodes or subsequent radiation therapy in breast cancer treatment. Management of lymphedema remains a clinproblem. In adult lymphangiogenesis, VEGF-C has been shown to be a specific mitogen for lymphatic endothelial cells (LEC) via the VEGF-3 receptor. Anhas recently been shown to be required for proper lymphatic development via the Tie 2 receptor. Objective: In our model we incorporated into alginate gAng-2 and VEGF-C to promote lymphoangiogenesis by stimulating LEC proliferation and migration. Methods: Sterile alginate gels with Ang-2 (2µg/ml) aVEGF-C (200ng/ml) were tested in vitro and in vivo for proliferation and migration. Results: By adding Ang-2 to the VEGF-C alginate gels, LEC proliferatand migration increased, when compared to VEGF-C alginate gels. These gels were also compared in our in vivo mouse tail lymphedema model asucceeded in reducing lymphedema and restoring lymphatic function in our acute lymphedema animals. However, in our chronic lymphedema model, we wunable to induce the atrophic adipose tissue changes seen clinically in secondary lymphedema. Thus, unable to determine if this therapeutic application wohave restored lymphatic function. Conclusions: Our in vitro results demonstrate that alginate gels are an effective delivery system of Ang-2 and VEGF-Cwhich new tubular structures are formed resembling lymphatic vessels. In vivo, for the acute lymphedema animals, this therapy was successful, unfortunatfor the chronic lymphedema animals this was not to be the case. Further studies are required to evaluate this new therapy and its capability in restorlymphatic function in secondary chronic lymphedema in a different animal model.

15. SUBJECT TERMS
Breast Cancer

16. SECURITY CLASSIFICATION OF:				18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	16	19b. TELEPHONE NUMBER *(include area code)*

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Introduction:

Breast carcinoma continues to be the most frequently occurring carcinoma in women. Presently, approximately one in every eight women will develop the disease.[1] Great strides have been made in the treatment of breast carcinoma that have reduced the risk of recurrence and improved survival rates.
The removal of axillary lymph nodes has been an integral part of breast carcinoma treatment since the end of the last century.[2] Axillary lymph nodes are removed to provide accurate information for staging, to accomplish local control, and for prognosis in order to plan adjunctive, systemic therapy. The status of the axillary lymph nodes remains the single most important predictor of survival.[3] Therefore, axillary treatment surgically and/or with radiotherapy is still an essential component in the management of most patients with invasive breast carcinoma.[4-6]

Chronic edema of the arm, commonly called Lymphedema or post-mastectomy edema (PME), was described first as a side effect of mastectomy operations by Halsted in 1921.[7] Although there has been a trend toward more conservative surgery and greater use of radiotherapy, PME remains a common iatrogenic problem (*Figure1*).

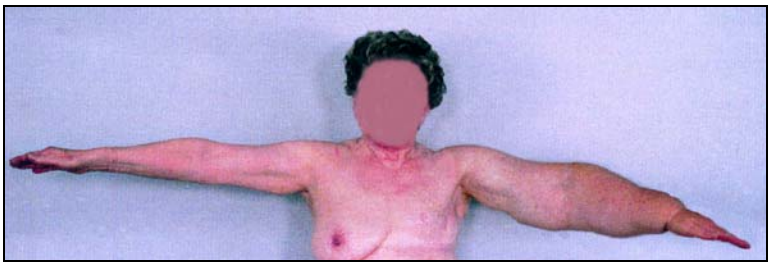


Figure 1: Photograph of Secondary Lymphedema due to Breast Cancer treatment. Lymph stagnation within the left arm's adipose tissue and subsequent swelling (edema).

Edema represents an increase in interstitial fluid volume sufficient to manifest with swelling. Any edema, whatever the underlying cause, is due to an imbalance between capillary filtration and lymph drainage.[8] Most examples of limb edema are caused by an increase in capillary filtration, overwhelming lymph drainage capacity. Lymphedema, however, strictly occurs when swelling is due to a failure of lymph drainage in circumstances in which capillary filtration is not increased.

The lymphatic system is a one-way drainage route designed to rid the "tissues" of unwanted material and excess fluid. It therefore represents a waste route and overflow pipe, with its essential function being to return to the blood vascular compartment protein, colloids, and particulate matter too large to reenter the blood compartment directly [9] *(Figure 2)*. Two types of lymphatic vessels exist: First, the smaller initial lymphatic, which includes the smallest lymphatic capillary and the larger pre-collector vessel, and second, the collecting lymphatic vessel into which the pre-collectors drain.[10]

The collecting lymphatics are the main limb lymphatic vessels that provide the afferent flow to the lymph nodes. They behave like a series of smooth muscle hearts that are responsible for the propulsion of lymph centripetally.[11] Intrinsic pumping of collecting vessels is essential motor for

lymph propulsion. For initial lymphatics, however, flow of interstitial fluid and macromolecules is caused by intermittent changes in hydrostatic and oncotic pressures locally.[12] Deformation or movement of the tissues by surface pressure or underlying muscle contractions and by other

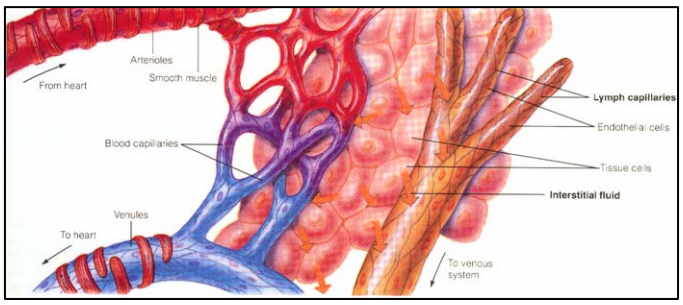


Figure 2

Figure 2:
Schematic representation of lymphatic vessel, arteriole and venule in the interstitial space. Proteins unable to be incorporated into the lymphatic circulation accumulate in the adipose tissue causing edema.

contractile structures, such as arterioles, causes expansion or compression of the initial lymphatics. The compression forces lymph along initial lymphatics. Valves in both, initial and collecting lymphatics ensure that flow is unidirectional. Lymphedema arises when an intrinsic fault develops within the lymph-conducting pathways (primary lymphedema) or when damage occurs from one or more factors originating outside the lymphatic system, such as surgical removal of lymph nodes (secondary lymphedema).

Lymphedema therefore, is a disease characterized by abnormal collections of fluid and proteins within the interstitial space. Treatment options for this debilitating condition have included drug therapy, physical therapy, and surgical approaches that have yielded limited success. Unfortunately, for lymphedema today, treatment options are all palliative, for there is **no effective treatment** that could offer a permanent cure to this disease.

Secondary Lymphedema resulting after iatrogenic, surgical disruption of the lymphatic vessels in breast cancer surgery, has continued to be neglected in the U.S. in spite of the fact that it has now become an acceptable diagnosis (ICD9-457.0).[13] Swelling of the upper limb, constitutes the most invalidating complication of breast carcinoma treatment. Untreated, upper extremity lymphedema predisposes women to the development of severe acute or chronic infections with limitations on functioning and serious disturbances in a patient's quality of life. Psychological distress (disfigurement), depression and social inhibition.[14-15]

It is estimated that 15-20% of the 2 million breast cancer survivors, are living currently with post-treatment lymphedema.[16-17] The swollen arm, which can be as much as twice the normal size, is disfiguring and commonly causes functional impairment, psychosocial maladjustment and psychological morbidity.[18] Added to the physical symptoms that patients must cope with, is the pain caused unintentionally by clinicians that, interested in carcinoma recurrence, trivialize the non-lethal nature of lymphedema.

Body:

Vascular Endothelium Growth Factor (VEGF) is produced by many different cell types both in tissue culture and in vivo. It binds to plasma membrane receptors on endothelial cells (EC) only with an extra cellular transmembrane glycoprotein linked to an intracellular tyrosine kinase domain, thus VEGF is an EC specific mitogen.

VEGF-C is a 38 kD homodimeric glycoprotein that is commercially available. This recombinant human VEGF-C is from a DNA sequence encoding the 165 amino acid residue variant of human VEGF expressed in Sf 21 insect cells using a baculovirus expression system. The product is lipolized from a sterile-filtered solution in 30% acotonitrile plus 0.1% TFA containing 50μg of cytokine. **VEGF-C** is of particular interest because it is a specific mitogen for **lymphatic endothelium** in adult tissues.

Angiopoietin-2 is a ligand for the endothelial Tie-2 receptor tyrosine kinase, it has a dual function in the processes of postnatal angiogenesis and vascular remodeling. Also **Ang-2** signals are required for the proper development and function of **lymphatic vessels.** Recent data also suggest that the roles of Ang-2 and VGEF-C in the lymphatic vessel development are analogous too the roles of Ang-1 and VEGF in blood vessel development. Thus, Ang-2 is the first endothelial cell-specific growth factor demonstrated to function in vessel formation or regression depending on the tissue context.[19] In combination with VEGF-C, Ang-2 potentiates its effect.

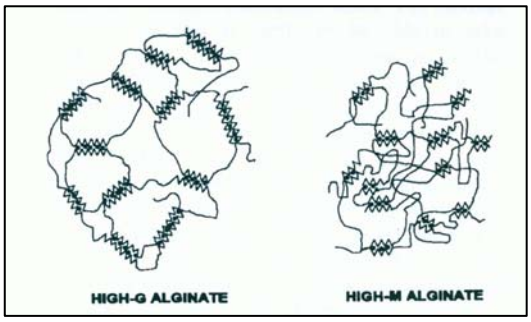
Tissue engineering is an interdisciplinary field that incorporates principles of engineering and polymer chemistry into the biological sciences, in efforts to develop biological substitutes for failed tissues and organs. It is a new and rapidly expanding field, in which the techniques are being developed for culturing or promoting regeneration of a variety of tissues, both in vitro and in vivo using polymer “scaffolds” to support tissue growth.[20]

Unlike the solid polymer systems currently used to create a cell-polymer construct, a liquid support matrix that polymerizes to a gel would have the potential for injectable delivery, which would be much less invasive than open implantation. **Alginate hydrogels** have been increasingly important in biotechnology applications, such as tissue engineering, artificial organs and as drug carriers.[21] Delivery rate can be predetermined and its local, rather than systemic administration be of great advantage (Alginates are approved by Food and Drug Administration for human use).

Alginate refers to a family of polyanionic copolymers derived from brown sea algae and comprising 1,4-linked β-D-manuronic (M) and α-L-glucoronic (G) acid residues in varying proportions. The fact that G and M are C5 epimers results in a switch-over of the monomer chair conformation, giving rise to all four possible glycosidic linkages and at the molecular level, large effects like cavity formations between G residues are observed. It has been shown that the occurrence of G and M within the alginate molecule is block-wise and not random.[22,23]

There is a direct dependence between the mechanical strength of an alginate gel and the porosity of the gel network. When gels are made from alginate rich in glucoronic (G) acid residues,

higher moduli are obtained compared to gels made from alginates less enriched in G residues, and also higher diffusion rates. *(Figure 3*). Gelling properties of alginate are a function of the M/G composition and the sequential structure of M and G along the alginate chain (*Figure 3*).



In general terms, an increase in G content (measured as $G_{n>1}$) as well as in molecular weight will give a stronger gel. In contrast to most gelling polysaccharides, alginate gels have the particular feature of being "cold setting"'. This implies that the setting of alginate gel is more-or-less independent of temperature.

Figure 3

Alginate gel incorporation: Essentially there are two main methods for the preparation of alginate gels: The dialysis/diffusion method and internal geleation method. In the dialysis/diffusion method (diffusion setting) gelling ions are allowed to diffuse into the alginate solution. In our study we will incorporated sterile G (50%) and M (50%) copolymers of alginate to a solution with Potassium Phosphate and Sodium Chloride (0.1 M K_2HPO_4 and 0.135 M NaCl, pH of 7.4), which was previously sterilized by autoclave. A 1.0% Sodium Alginate solution will then be mixed with 0.2 gm of $CaSO_4$ per milliliter, this solution will be placed in ice prior to extrusion. Because of all the different characteristics previously mentioned we decided to produce an alginate gel that would have the best properties of both, the G and M copolymers. Thus providing a gel that would be highly porous and relatively strong. Previously expanded EPC's which have been induce to differentiate into LEC by the growth factors, VEGF-C and Ang-2, will be incorporated into this gel for injection.

Animal Model for LEC harvest:

We continue to use the same animal model described last year to harvest the LEC to grow in culture to use in our in vitro studies.

Preliminary in vitro studies were carried out to determine the ideal Ang-2 concentration to be incorporated into the alginate gels. *In vitro* culture with LEC and three different concentrations (as recommended by Regeneron): 0.22 μg/ml, 0.67 μg/ml and 2 μg/ml of Ang-2 reveled that the proliferative and migratory response of the LEC was greater at 2 μg/ml when compared to the other two concentrations.

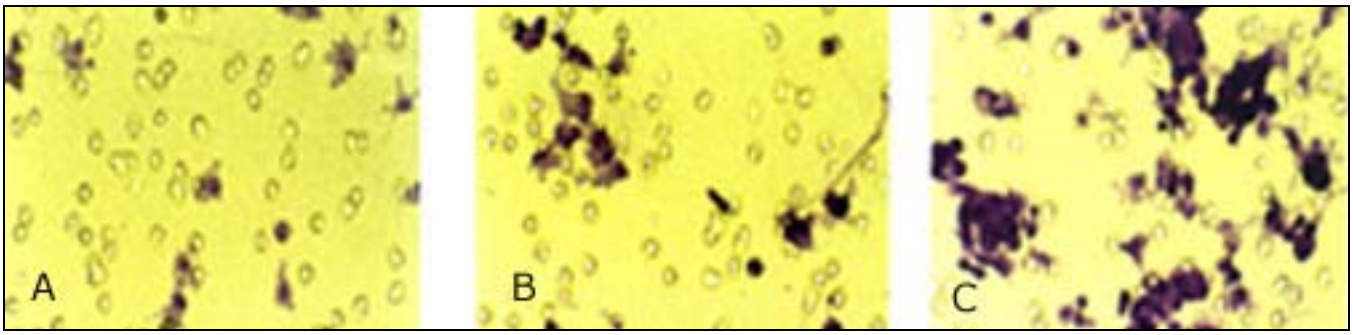


Figure 4. Photograph of in vitro Proliferation and migration Assays of LEC in a Bodin chamber at 7 days. A) Ang-2 concentration of 0.22 μg/ml, B) Ang-2 concentration of 0.67 μg/ml, C) Ang-2 concentration of and 2 μg/ml.

With these initial results we determined that the concentrations of Ang-2 that should be incorporated into alginate gels should be the same as the ones above, and determine if the release of the growth factor from the alginate gel would result in the same or comparable proliferation and migration of LEC.

LEC's were cultivated with Ham's modified F12K medium for EC with ECGS. Cells were serum starved (from 10% to 1%FBS) prior to plating on the petri-dish with the alginate gels. Once again the ideal concentration for Ang-2 was that of 2 μg/ml, where the proliferation and migration of LEC was more profound.

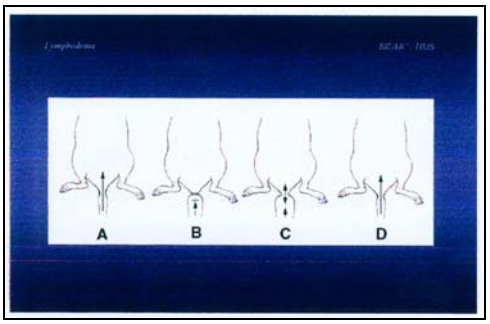
Alginate gels were then prepared to incorporate VEGF-C at a concentration of 200 ng/ml and Ang-2 at a concentration of 2 μg/ml. These gels will now be used *in vivo*, in the mouse tail animal model we have worked on.

Lymphedema animal model: A lymphedema mouse model was recently described in the literature.[24] This model provides a useful *in vivo* system, in which experimental treatments for lymphedema can be evaluated. We operated the tail of mice to cauterize the lymphatics and induce lymphedema (*Figure 5*).

In this model, hairless mice (SKH1/Charles River Laboratories, Pittsfield, NH) weighing between 20 are 30g were used. The hairless mice offer the advantage that lymphedema development and subsequent tail diameter measurements are easier to follow. Anesthesia and Analgesia: The animals were anesthetized by placing them inside an induction chamber with 1.5% isoflurane and 100% Oxigen (JA Webster Inc., Sterling, MA). Once the animals were anesthetized they were placed in a prone position and the head placed inside a conical mask to maintain anesthesia (0.8% Isoflurane and 100% Oxigen). Analgesia was maintained post-op and for the next 7 consecutive days by daily subcutaneous (SC) injections of 3.3 mg/Kg butorphanol.

Prior to start the surgical procedure, 0.10 ml of 5% blue dextran 2M (Sigma, St. Louis, MO) in saline is injected SC into the tip of the tail in order to identify the lymphatic vessels. Then, the operative site at the base of the tail is cleansed with 70% ethanol and povidine/iodine, and a

circumferential incision is made through the dermis to sever the superficial lymphatic network. The three deep lymphatic vessels located in the lateral and ventral aspects of the tail are isolated and destroyed by electrical cauterization. Finally, low cauterization is applied too the edges of the circumferential wound, which results in a 2-4 mm gap between the skin edges followed by secondary healing at the site *(Figure 5,6).*



Schematic of Lymphatic tail model.

A) Normal lymphatic flow.
B) Obliteration of tail lymphatics with subsequent lymphedema formation.
C) Lymphangiogenesis by transplantation (Injection) of Alginate gel with VEGF-C and Ang-2.
D) Restoration of normal lymphatic flow with subsequent lymphedema reabsorption.

Figure 5

Once the lymphedema of the tail has been developed, the transplantation (SC injection) of the alginate gel containing the growth factors, VEGF-C and Ang-2 was performed in an attempt to induce lymphatic vessel proliferation and regeneration of the lymphatic network previously destroyed. Subsequent Lymphocintography studies would confirm if lymphatic flow had been restored.

Last Phase of Research: *In Vivo* model.

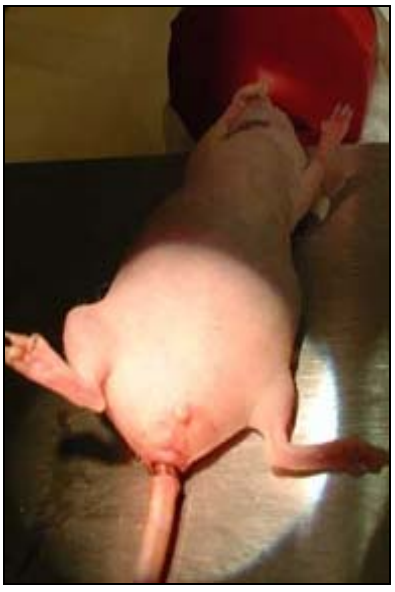


Figure 6

Figure 6:

Ang-2 & VEGF-C Alginate gels Transplantation Animal Model. All procedures were performed in accordance with the Animal Care Act and Use Committee. Hairless mice (Charles River Laboratories) age 8-10 weeks and weighing 20-30 g were anesthetized with 160 mg/kg pentobarbital IP. for operative transplant (injection) of the lymphedematous tails. A total of 24 animals were used in this study.

Acute Lymphedema *vs* Chronic Lymphedema: When using this lymphedema model, we found that to study the effect of our alginate gels in acute lymphedema (14 and 28 days) was possible *(Figure 7).* However, when we tried to maintain the model for a longer period of time, about 3 months it was very difficult to attain. The animals had to be subjected to a second or

even third "injury" procedure, and even in those cases we were not able to see the adipose atrophic changes that one sees in chronic lymphedema patients.

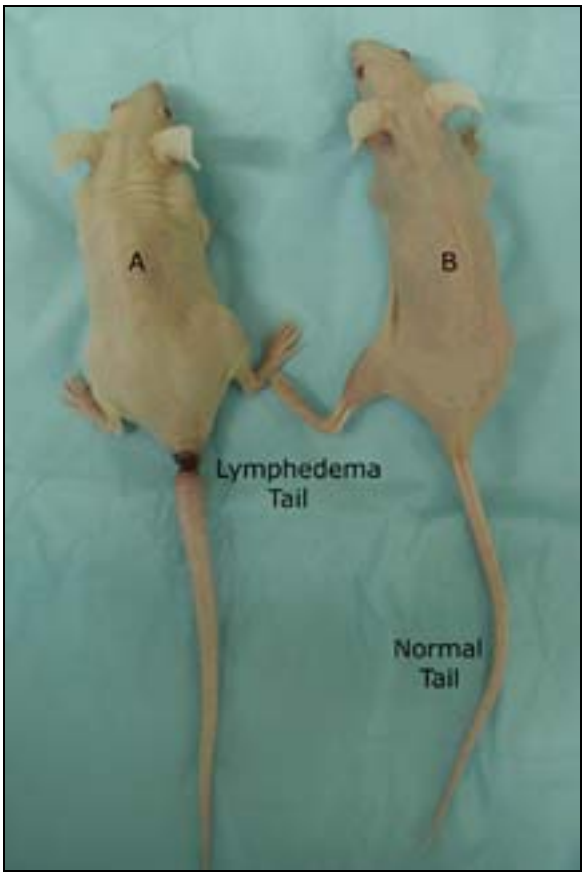


Figure 7: Photograph of Lymphedematous
Tail in the mouse (A), at 2 weeks.
The increase in diameter of the tail is more profound at a later time point 4 weeks.
When compared side-by-side to a normal (B) tail of a mouse, the Lymphedematos tail is also evident.
Alginate gels with Ang-2 & VEGF-C were injected at different time intervals (14
and 28 days) to evaluate lymphatic regeneration and restoration of lymphatic flow.

Figure 7

Physiological Assessment of Transplanted Animals. At 14 and 28 days after transplantation, Lymphocintography studies were conducted to assess lymphatic regeneration. Immediately before sacrifice, mice were injected with an overdose of pentobarbital.

In the control animals, the tail lymphatic circulation was interrupted, while the test, alginate gel (VEGF-C & Ang-2) were able to induce linphoangiogenesis and restore normal lymphatic flow through the mouse tail. *(Figure 8).*

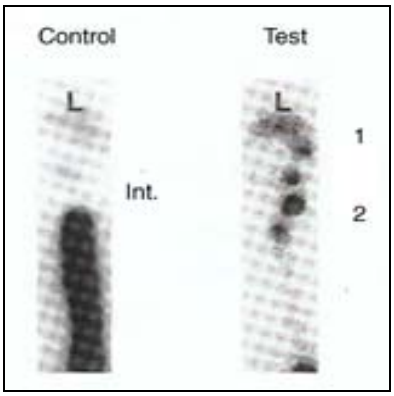


Figure 8

Figure 8:
On the left (Control), the lymphatic circulation is interrupted (Int.), while on the right (Test), the lymphatic circulation has been restored at 14 days after treatment with alginate gels.

L , Lymphatic flow
1 , Renal node
2 , Inguinal node

Histologic Assessment of Transplanted Animals. Tissue sections from the base of the Lymphedematous and healthy tails were performed. Tails were harvested on days 14 and 28 after transplantation. For conventional histology, tissues were fixed in formalin and embedded in paraffin. Cross sections of 6-µm thickness were mounted on glass slides, and stained with H&E (*Figure 9, 9a and 9b*)

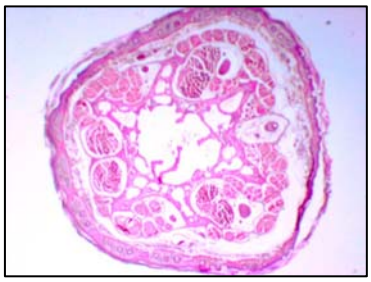


Figure 9

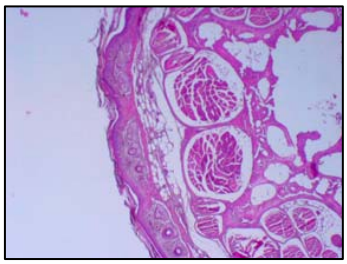


Figure 9a

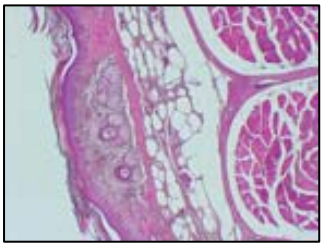


Figure 9b

Figure 9, 9a and 9b:
Cross section of mouse tail at the base. Figure 9 at 1X magnification. Figure 9a at 4X magnification and Figure 9b at 10 X magnification. Note that there are no atrophic changes in the adipose tissue and that there is lymphatic integrity.

Key Research Accomplishments:

Our Specific aims for this award were completed.

We were able to incorporate into our previous porous, biodegradable alginate gel with VEGF-C a second growth factor, Ang-2 to serve as a receptor agonist, thus enhance the VEGF-C mitogenic effect in promoting Lymphatic Endothelial Cell proliferation and migration *in vitro*.

We induced (surgically) lymphedema of the tail of mice to inject the VEGF-C and Ang-2 alginate gels to evaluate lymphoangiogenesis *in vivo*.

Reportable Outcomes:

Rat Thoracic Duct Cell line maintained.

Abstract submitted to the International Meeting on Experimental Biology 2003, San Diego, California. April 11-15, 2003 and was accepted for Oral presentation.

Abstract printed in the FASEB Journal. ABSTRACTS / Part II (Abstracts A456.1-A886.2) Volume 17, No.5, March 17, 2003.

AIVS 2005 Promoting lymphangiogenesis in vivo utilizing
Lech am Arlberg, Austria Alginate gels with VEGF-C and Angiopoietin-2
03/5-12/2005

Era of Hope 2005 Promoting lymphangiogenesis in vivo utilizing
Philadelphia, Pennsylvania Alginate gels with VEGF-C and Angiopoietin-2
06/8-11/2005

Publications:

Promoting lymphangiogenesis in vivo utilizing alginate gels with VEGF-C and Angiopoietin-2. Mauricio A. Contreras, MD and Sumner A. Slavin, MD. Proceedings Era of Hope: June 2005;66:P9-3

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Conclusions:

A biodegradable Alginate gel, may be an effective delivery system for sustained slow release of VEGF-C and Ang-2 to promote lymphangiogenesis in those instances where the integrity of the VEGF-3 and Tie-2 receptor of the LEC are intact.

Our lymphedema tail model proved to be a good model to induce acute lymphedema and test our alginate gels with growth factors VEGF-C and Ang-2. However, this is not a good model to assess chronic lymphedema.

To sustain the lymphedematous tail for prolonged periods of time (3 or more months) proved to be very difficult to obtain. Often times having to re-injure the lymphatics at the wound site. There were no atrophic adipose tissue changes as those seen clinically in patients with secondary lymphedema. Thus, the hopes to try out these gels in chronic lymphedema and subsequent restoration of lymphatic flow by inducing lymphoangiogenesis could not be assessed.
Perhaps a different animal model for lymphedema, where there is more adipose tissue on the underlying lymphatic bed, could yield the answers that we are seeking.

We will continue to look for a suitable model to try out this new therapy in the hopes that perhaps some day in the near future we could offer hope in treating this disease for which there is no cure.

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P9-1: ABOLISHING SYNCHRONOUS INTERACTION BETWEEN D-CYCLIN AND THE CDK IS A CONTROLLING FACTOR FOR TUNICAMYCIN-INDUCED UNFOLDED PROTEIN RESPONSE-MEDIATED CELL CYCLE ARREST IN CAPILLARY ENDOTHELIAL CELLS

Dipak K. Banerjee, Ph.D.,[1] Aurymar Sanchez, B.S.,[2] Subiman Saha, M.D.,[1] and Krishna Baksi, Ph.D.[2]
[1]Department of Biochemistry, School of Medicine, Medical Sciences Campus, University of Puerto Rico, San Juan, PR; [2]Department of Anatomy and Cell Biology, School of Medicine, Universidad Central del Caribe, Bayamon, PR
E-mail: dbanerjee@rcm.upr.edu

Angiogenesis is the formation of new capillaries from pre-existing vasculature by migration and proliferation of capillary endothelial cells. It is essential for normal growth and development, and is also a 'key step' in tumor growth and invasion. Angiogenesis from preexisting vasculature occurs in stages that orchestrate a network of cooperative interactions, significant components of which are endothelial cell migration, capillary budding, establishment of capillary loops, and neovascular remodeling. A large number of cytokines accelerate the process of angiogenesis by helping to control a switch in the angiogenic phenotype, which enhances glycosylation of proteins. Attachment of N-glycans to the protein core gives stability and increases the functional diversity of asparagine-linked (N-linked) glycoproteins. The process begins in the endoplasmic reticulum (ER) upon initiation with the assembly of Glc3Man9GlcNAc2-PP-Dolichol (lipid-linked oligosaccharide, LLO). Our objective has been to regulate the LLO biosynthesis in a non-transformed capillary endothelial cell line and study its influence on the angiogenic process. Treatment of these cells with a GlcNAc-1 phosphate transferase inhibitor, tunicamycin (an 840-dalton glucosamine-containing pyrimidine nucleoside) resulted in down–regulation of LLO biosynthesis, and differential expression of N-glycans on the cell surface. As a result the cell cycle is arrested in G1. Light microscopic analysis indicated cell shrinkage, loss of membrane contact with neighboring cells, apparent compaction of nuclei showing condensed pyknotic appearance and membrane fragmentation. The response was time- and concentration-dependent and could not be reversed by a protein synthesis inhibitor (cycloheximide). High expression of ER chaperones (i.e., Bip/GRP-78 and GRP-94) indicated the presence of an "ER stress". Analysis of protein expression by Western Blotting exhibited a reduced expression of D2 cyclin, cdk4 and the Bcl-2. Increased p53 expression and its downstream regulator p21WAF1/Cip1 confirmed the cell growth arrest in G1. Kinetic studies with D2 cyclin, and cdk4 expression indicated asynchronous interaction between these two cell cycle regulatory partners. Reduced expression of MMP1 in tunicamycin-treated cells suggested weakening of invasiveness. We therefore concluded that lack of a quantitative interaction between the D-cyclin and the cdk is a controlling factor for the tunicamycin-induced unfolded protein response-mediated cell cycle arrest in capillary endothelial cells. This research is highly significant because it opens a previously untapped territory for developing new generation anti-angiogenic therapeutics against breast tumor growth.

The U.S. Army Medical Research and Materiel Command under DAMD17-03-1-0754 supported this work.

P9-2: A CRITICAL ROLE OF EPHA2 RECEPTOR TYROSINE KINASE IN BREAST TUMOR ANGIOGENESIS AND METASTASIS

Dana Brantley-Sieders, Wei Bin Fang, Donna Hicks, and Jin Chen
Vanderbilt-Ingram Cancer Center, Department of Medicine and Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN
E-mail: jin.chen@vanderbilt.edu

Breast tumor interactions with the host environment resembles "seed and soil" in which the host environment is ideally suited to foster growth and malignant progression. Studies in the past have largely focused on molecular determinants in "seed" and "soil" separately. However, a single molecule that functions in both "seed" and "soil" might provide a better target for therapeutic intervention in cancer. EphA2 receptor tyrosine kinase is a novel candidate for such a target, since EphA2 is overexpressed in both breast tumor cells and blood vessel endothelial cells in the host environment in more than 70% of clinical breast cancer specimens. In addition, high level of EphA2 expression consistently correlates with high degree of breast tumor malignancy.

During the period supported by the DOD grant, we have been focusing on the role of EphA2 receptor in breast tumor angiogenesis. To determine the role of EphA2 receptor in tumor neovascular growth, we initially blocked A-class Eph receptor tyrosine kinase activation by soluble EphA2-Fc or EphA3-Fc receptors. These soluble receptors inhibited 4T1 mammary tumor progression in vivo, providing a first functional evidence of EphA receptor involvement in breast tumor angiogeneis. Our results were subsequently confirmed in several other studies in a number of animal tumor models.

To determine the specific role of EphA2 receptor in tumor angiogenesis, we characterized EphA2-deficient mice. EphA2-null endothelial cells (ECs) fail to undergo vascular assembly and migration in vitro and angiogenesis in vivo, and are defective in PI3K-dependent Rac1 GTPase activation in response to ligand stimulation. Loss of EphA2 receptor in the host significantly inhibited tumor volume, neovascularization and metastasis of orthotopically grafted 4T1 mammary tumors. In addition, EphA2-deficient ECs fail to migrate, coalesce, and incorporate into tumor blood vessels when co-transplanted with 4T1 tumor cells, indicating an EC-specific defect in tumor neovascularization. Taken together, these data suggest that host EphA2 receptor function is required in the tumor microenvironment for tumor angiogenesis and metastatic progression

Our findings are novel and significant in several respects. First, our data presented here, together with studies from other laboratories, indicate that EphA2 plays a key role in breast tumor progression through both tumor and host-dependent mechanisms. Because this single factor influences tumor progression in both tumor cells and tumor blood vessels, EphA2 receptor is an attractive target for the development of new treatment for breast cancer. Second, EphA2 receptor appears not to be required for embryonic development, but is critical in adult angiogenesis and cancer and thus targeted inhibition of EphA2 receptor shall have limited side effects to normal tissue. Finally, the new mouse model of EphA2 deficient mice provides a novel tool to dissect the function of EphA2 in tumor cells and tumor endothelium in vivo in clinically relevant breast cancer models.

The U.S. Army Medical Research and Materiel Command under DAMD17-02-1-0604 supported this work.

P9-3: PROMOTING LYMPHANGIOGENESIS IN VIVO UTILIZING ALGINATE GELS WITH VEGF-C AND ANG-2

Mauricio A. Contreras, M.D., and Sumner A. Slavin, M.D.
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA
E-mail: mcontrer@bidmc.harvard.edu

Secondary lymphedema is an abnormal swelling, in which lymph production exceeds drainage capabilities. This occurs as a result of lymphatic vessel destruction during the removal of lymph nodes or subsequent radiation therapy in breast cancer treatment, as well as, obstruction of the lymphatic vessels in filarial infection or trauma.

Management of lymphedema remains a clinical problem. Restoration of the lymph-transporting capacity is the only therapy that would deal directly with the cause of lymphedema.

Vascular Endothelium Growth Factor (VEGF) and the Angiopoietins (Ang) work in complementary and coordinated fashion during development of the lymphatic vasculature. In adult lymphoangiogenesis, VEGF-C has been shown to be a specific mitogen for lymphatic endothelial cells (LEC) via the VEGF-3 receptor. Ang-2 has recently been shown to be required for proper lymphatic development via the Tie 2 receptor.

In our model, we incorporated into alginate gels, VEGF-C and Ang-2 to promote lymphoangiogenesis by stimulating LEC proliferation and migration. We tested these gels in our in vivo mouse tail lymphedema model and succeeded to improve lymphatic function by reducing mouse tail lymphedema.

Further studies are required to evaluate the safety and possibility of a therapeutic application.

It is estimated that 15-20 % of the 2 million breast carcinoma survivors, are currently living with post-treatment lymphedema. The swollen arm, which can be as much as twice the normal size, is disfiguring and commonly causes functional impairment, psychosocial maladjustment and psychological morbidity.

Original work supported by the U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0152 and current work supported by DOD-BCRP.

P9-4: THE ROLE OF EPHA2 RECEPTOR TYROSINE KINASE IN HOST-TUMOR INTERACTIONS

Dana M. Brantley-Sieders,[1] Wei Bin Fang,[2] Yu Shyr,[3] and Jin Chen[1,2]
Departments of [1]Medicine, [2]Cancer Biology, and [3]Biostatistics, Vanderbilt University School of Medicine, Nashville, TN
E-mail: dana.brantley@vanderbilt.edu, wei.b.fang@vanderbilt.edu

The Eph family of receptor tyrosine kinases (RTKs) and their membrane-tethered ligands, known as ephrins, regulate a diverse array of biological processes including cellular adhesion, migration, and tissue boundary formation. Several members of this family regulate vascular remodeling during embryogenesis, and more recently these molecules have been associated with tumor neovascularization. In particular, overexpression of EphA2 RTK and its principle ligand, ephrin-A1, has been observed in a wide variety of human cancers and associated endothelium, including breast cancer. Functionally, we and others demonstrated that soluble receptor-mediated inhibition of multiple Eph RTKs impairs tumor angiogenesis and progression in vivo, and targeted disruption of EphA2 RTK specifically inhibits ephrin-A1-mediated vascular remodeling in vivo and endothelial cell migration in vitro. Here, we demonstrate that host EphA2-deficiency impairs breast tumor angiogenesis and progression in vivo. 4T1 breast